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Research

Rhizodeposition stimulated by elevated CO_2 in a semiarid grassland

Elise Pendall[1], Arvin R. Mosier[2] and Jack A. Morgan[3]

[1]Department of Botany, University of Wyoming, Laramie, WY 82071, USA; [2]USDA-ARS SPN Unit, 2150 Center Ave., Fort Collins, CO 80526, USA; [3]USDA-ARS Crops Research Laboratory, 1701 Center Ave., Fort Collins, CO 80526, USA

Author for correspondence:
Elise Pendall
Tel: +1 307 766 6293
Fax: +1 307 766 2851
Email: Pendall@uwyo.edu

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Summary

• Rhizodeposition, or the addition of C from roots to soil C pools, is expected to increase if net primary production is stimulated and some excess C is allocated below-ground. We investigated the effects of 5 yrs of elevated CO_2 on below-ground C dynamics in a native, C_3–C_4 grassland ecosystem in Colorado, USA.

• Cylinder harvests following each growing season and monolith excavation at the end of the experiment provided data on root biomass, root C : N ratios, and root and soil $\delta^{13}C$ values. We applied an isotopic mixing model to quantify new soil C inputs on elevated and ambient CO_2 treatments.

• Root biomass increased by 23% and root C : N ratios increased by 26% after 5 yrs of elevated CO_2. Species-specific differences were found in root residence times, which ranged from 6 to 8 yrs.

• Rhizodeposition was roughly doubled in elevated compared with ambient CO_2 chambers, at 83 ± 16 versus 35 ± 9 g C m^{-2} yr^{-1} over the last 4 yrs of the experiment (*t*-test, $P = 0.006$). Net C sequestration will depend on how decomposition rates are altered by elevated CO_2.

Key words: elevated CO_2, soil carbon, root biomass, stable isotopes, $^{13}C/^{12}C$, roots, C : N ratio, rhizodeposition.

 162: 447–458

Introduction

Elevated CO_2 stimulates carbon cycling in many ecosystems, but how much additional carbon may be stored in long-lived pools such as soils or wood has been difficult to assess. Perennial grassland soils may store carbon at higher rates when exposed to double CO_2 concentrations (Jastrow *et al.*, 2000), although decomposition of soil organic matter (SOM) has been shown to increase (Pendall *et al.*, 2003). Because up to 80% of the biomass and at least 50% of net primary production (NPP) can occur below-ground in grasslands, changes in rhizodeposition will have a large impact on C cyling in these ecosystems (Milchunas & Lauenroth, 2001).

Rhizodeposition is soil C derived from turnover of fine roots, root hairs and mycorrhizas, secretion of soluble root exudates, and turnover of rhizosphere-associated microbial biomass. This C increment is generally too small to measure on an annual basis using total C analysis. However, in experiments where growing plants and pre-existing SOM have contrasting $\delta^{13}C$ values, rhizodeposition can be quantified as the portion of soil C derived from the newly labelled plant C, whether that label results from a C_3–C_4 plant community shift, or from an imposed $^{13}CO_2$ source (Paterson *et al.*, 1997; Leavitt *et al.*, 2001). Rhizodeposition may account for up to 40% of assimilated C (Paterson *et al.*, 1997), although much of this is rapidly metabolized by the rhizosphere microbial community (Kuzyakov, 2002). The remaining C inputs to SOM can be quantified using isotope techniques.

The fate of C allocated below-ground under elevated CO_2 will depend on its decomposability, which is partly related to tissue N concentration. Root N concentration has been shown to decrease by 10–25% under elevated CO_2, as C accumulation dilutes N pools (Curtis *et al.*, 1990; Berntson & Bazzaz, 1997; King *et al.*, 1997; Cotrufo *et al.*, 1998; Rogers *et al.*, 1999; Pregitzer *et al.*, 2000). In perennial grasslands like the N-limited shortgrass steppe, autumn senescence of leaves results in the translocation of N to perennial root systems for storage (Charley, 1977). For these reasons, the C : N ratios of

below-ground biomass are of interest when evaluating the potential for altered C cycling under elevated CO_2.

In a Colorado shortgrass steppe open-top chamber experiment, twice-ambient atmospheric CO_2 concentrations stimulated NPP above-ground by 15–35% over 5 yrs (Morgan *et al.*, 2001, 2004). Our goal was to evaluate the changes in root biomass and C : N ratios over 5 yrs, and to quantify the input rate of new soil C, or rhizodeposition, in both ambient and elevated CO_2 treatments.

Methods

Experimental conditions

An elevated CO_2 experiment was conducted in the shortgrass steppe region of north-eastern Colorado, at the USDA-ARS Central Plains Experimental Range (CPER; latitude 40°40′ N, longitude 104°45′ W), about 55 km northeast of Fort Collins. The most abundant species at the study site were the C_4 grass, *Bouteloua gracilis* (H.B.K.) Lag. (blue grama), and the C_3 grasses *Stipa comata* Trin and Rupr. (needle-and-thread grass) and *Pascopyrum smithii* (Rydb.) A. Love (western wheatgrass). Root biomass (including crowns) is responsible for up to 70% of NPP in this ecosystem (Milchunas & Lauenroth, 2001). Soils were weakly developed fine sandy loams in the Remmit series (Ustollic Camborthids), and were carbonate-free above 40 cm depth.

From 1997 to 2001, open-top chambers (OTC; 4.5 m diameter) were used to evaluate the effects of CO_2 on the shortgrass steppe ecosystem, with 3 replicate chambers at ambient (AC, 360 ± 20 ppmv) and elevated (EC, 720 ± 20 ppmv) CO_2. Three nonchambered (NC) plots of the same area allowed evaluation of chamber effects. Chambers were placed on the plots before growth started in late March or early April, and removed at the end of the growing season in late October. Blowers with ambient or elevated CO_2 ran continuously. The experimental and chamber design was described in detail by Morgan *et al.* (2001).

An isotopic disequilibrium between growing plants and SOM of 4–5‰ on AC and NC plots may have been caused by increased C_3 biomass following a reduction in grazing at our site (Milchunas *et al.*, 1988). The disequilibrium might also have been partly due to fractionation by mycorrhizas (Henn & Chapela, 2000) or microbial decomposition (Mary *et al.*, 1992). The tank gas used to double the atmospheric CO_2 concentration had an approximate $\delta^{13}C$ value of −40‰ during the experiment, which produced air in the elevated chambers of −24.7 ± 1.4‰, which compared with background air $\delta^{13}C$ values of −8.1 ± 0.2‰ (Pendall *et al.*, 2003). The isotopic disequilibrium imposed by the EC treatment was thus about 16‰.

Root and crown biomass and C : N ratios

Eighteen months before the start of the experiment (November, 1995), ten 20-cm diameter steel cylinders, 60-cm long, were driven into the soil in each experimental plot to within 1-cm of the soil surface. No litter or water was collected in the cylinders, and above-ground vegetation growing in the cylinders appeared no different from outside the cylinders. At the end of each growing season (1997–2001), two cylinders were removed from each plot, cut open, and soil and roots were removed quantitatively at the following depth intervals: 0–5, 5–10, 10–20, 20–30, 30–40 and 40–60 cm. Weight and gravel content were determined for each interval for bulk density estimation. Most roots were removed immediately after sampling, with additional root picking done by hand on dried subsamples. The same technicians performed the root picking in all 5 yrs of the experiment. Crowns were clipped from the roots at the soil surface, and above-ground biomass was removed. Roots and crowns were pooled by cylinder and not identified to species. Subsamples of roots and crowns were ashed at 450°C to determine the ash-free biomass. Additionally, subsamples were analysed in an elemental analyser for C and N determination (Europa Scientific ANCA-NT System Solid/Liquid Preparation Module, PDZ Europa Ltd., Crewe, UK); precision was 0.1% for both elements. Repeated measures analysis of variance (ANOVAR) with CO_2 treatment and depth as fixed effects and year as the repeated measure was used to examine changes in root biomass and C : N ratios over the 5-yr experiment. Fisher's protected least significant difference was used for posthoc mean comparison.

At the end of the experiment, 9 root monoliths were excavated by backhoe (one per plot). One monolith, 20 × 100 cm and 75 cm deep, was removed from each experimental plot from the area that had been reserved for above-ground biomass harvests, and so had remained undisturbed below-ground for the duration. We assumed that experimental removal of the above-ground biomass at the end of each growing season was similar to what would have been removed by herbivory or fire in this ecosystem, and that below-ground herbivory was similar among treatments. For each monolith, entire root systems of several individual plants were separated, so that the isotopic composition of roots with depth could be determined for the three main species (*S. comata*, *P. smithii*, and *B. gracilis*). This was the only time root samples were collected by species; annual cylinder samples pooled all species. $\delta^{13}C$ value of roots from the 3 main species were determined by elemental analyser-mass spectrometry (EA-MS; Europa Scientific 20–20 Stable Isotope Analyser). A two-part mixing model (Eqn 1, below) was used to estimate the new C added to roots of each species in the elevated CO_2 chambers, using the average $\delta^{13}C$ value of C_3 or C_4 plants from EC plots over the 5 yrs for the 'new' end-member, and NC root $\delta^{13}C$ values for the 'old' end members for each species. The proportion of new root C added over 5 yrs was simply divided by 5 to estimate the annual proportional input rate, the inverse of which is the residence time. This approach assumed steady-state biomass and did not account for storage of pre-label nonstructural carbohydrates (Luo, 2003).

Rhizodeposition or 'new' soil carbon

Soil samples from the cylinders were picked by hand to remove visible roots, and then ground to a fine powder for analysis by EA-MS for C content and $\delta^{13}C$ value, with precision of 0.1% and 0.2‰, respectively. Soils were carbonate-free to a depth of 40 cm, and we therefore did not acidify the samples. The fraction of C that has been contributed by rhizodeposition (F_{new}) was determined using the two-part mixing model described by Balesdent *et al.* (1988):

$$\delta^{13}C_{SOC} = F_{new}(\delta^{13}C_{new}) + (1 - F_{new})(\delta^{13}C_{old}) \quad \text{Eqn 1}$$

where $\delta^{13}C_{SOC}$ is $\delta^{13}C$ of soil organic C (SOC) at a given depth interval in AC or EC soils, $\delta^{13}C_{new}$ refers to the 'new' end-member (defined below), and $\delta^{13}C_{old}$ is the $\delta^{13}C$ value of nonchambered SOC from the corresponding depth interval. To solve for the fraction (proportion) of new C in any layer, Equation (1) can be rearranged to:

$$F_{new} = (\delta^{13}C_{SOC} - \delta^{13}C_{old})/(\delta^{13}C_{new} - \delta^{13}C_{old}) \quad \text{Eqn 2}$$

The 'new' $\delta^{13}C$ end member was determined as follows. Above-ground biomass clipping of 1.5 m^2 subplots was done annually at peak green biomass (July). Leaves and stems were separated by species, dried, ground and analysed for analysis of C content and $\delta^{13}C$ value by EA-MS. The leaf $\delta^{13}C$ values were weighted by above-ground biomass amounts, after adding 1.4‰ to the leaf values to account for the average offset between leaves and roots in NC plots, for the final 'new' C end member signatures ($\delta^{13}C_{new}$; Table 3). The three main species are associated with endomycorrhizas, making it impossible to distinguish C contributions from roots versus mycorrhizas. July harvest samples were assumed to represent the proportion of C_3 and C_4 species for the entire growing season; leaf samples harvested at the end of the season (peak total biomass) were not easily separated by species. The three dominant species maintain similar shoot : root ratios at ambient and elevated CO_2, and we therefore assumed that $C_3 : C_4$ ratios in roots were the same as in above-ground biomass (Morgan *et al.*, 1994). We also assumed that $\delta^{13}C_{new}$ was constant within a season and with depth in the profile. Periodic $\delta^{13}C$ measurements of *S. comata* on NC plots through the dry 2001 growing season suggested that seasonal $\delta^{13}C$ dynamics were minimal (standard error < 0.2‰, $n = 8$). C inputs from rhizodeposition can also include turnover of mycorrhizas; in the short grass steppe, all root symbionts were endomycorrhizas, and therefore our root analyses of C : N and $\delta^{13}C$ included the mycorrhizal community.

F_{new} was multiplied by the total mass of C in each horizon or depth increment to calculate rhizodeposition going into each horizon on a mass basis using bulk density estimates. Whole-profile rhizodeposition was calculated by summing the depth increments. Uncertainties in estimating rhizodeposition were determined by accounting for variability in all of the components, including $\delta^{13}C_{SOC}$, $\delta^{13}C_{old}$, and $\delta^{13}C_{new}$, following a first-order Taylor series approach (Phillips & Gregg, 2001). Uncertainty estimates for $\delta^{13}C_{new}$ included standard errors of percentage biomass and $\delta^{13}C$ values of C_3 and C_4 grasses, as well as the standard error for the average root $\delta^{13}C$. We further corrected for covariance of C content and $\delta^{13}C$ values among soil depth intervals when calculating the standard error for the whole profile (Donald L. Phillips, personal communication).

Results

Root biomass and C : N ratios

The root monoliths excavated after the 5-yr experiment appeared to contain more biomass in the elevated CO_2 than ambient CO_2 chambers, but the trend was not significant. High variability of root biomass within each treatment was observed, despite that each monolith contained ~250 kg of soil. On average, root biomass per unit area in monolith samples was not significantly different from that in cylinders harvested in 2001.

In cylinders, which were excavated at the end of each growing season, root biomass was significantly increased by elevated CO_2 ($P = 0.006$) and showed significant interannual variability ($P < 0.0001$; Table 1; Fig. 1a). After increasing from 1997 to 1998 ($P = 0.008$), root biomass declined in 1999 ($P < 0.0001$) and again in 2000 ($P = 0.028$). We investigated the marginally significant year by CO_2 interaction ($P = 0.066$; Table 1) and found that in 1998, root biomass increased under elevated CO_2 (EC > AC, $P = 0.001$) but was reduced by the presence of open-top chambers (NC > AC, $P = 0.013$). The enhancement of root biomass by EC was therefore stronger than the reduction caused by the chamber effect. Both the CO_2 and chamber effects went away in 1999 and 2000 (Fig. 1a). In 2001, significant CO_2 effects reappeared (EC > AC, $P = 0.014$), and the chamber effect diminished below significant levels. Crown biomass is clearly an important portion of the ecosystem C in the shortgrass

Table 1 Results of repeated measures analysis of variance for root biomass (ash corrected), for 1997–2001, with CO_2 and Depth as main effects and Year as the repeated measure

Effect	DF	*P*-Value
CO_2	2	0.0063
Depth	5	< 0.0001
$CO_2 \times$ Depth	10	0.6095
Year	4	< 0.0001
Year $\times$ CO_2	8	0.0661
Year $\times$ Depth	20	< 0.0001
Year $\times$ $CO_2 \times$ Depth	40	0.3535

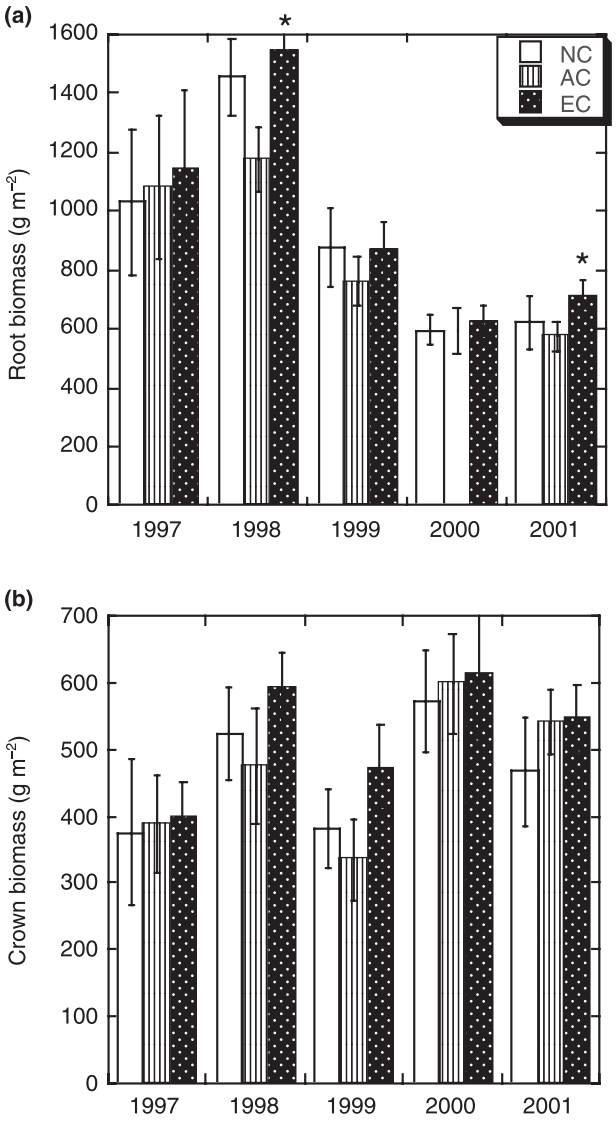

Fig. 1 Root and crown biomass (ash-corrected) in nonchambered (NC), ambient CO_2 (AC) and elevated CO_2 (EC) chambers over the 5-yr experiment. Asterisks show years when EC was significantly different from AC ($P < 0.05$). Error bars show SE of the mean of the total biomass. (a) Total root biomass to 60-cm depth. (b) Crown biomass (note different scale). No treatment differences were found in crown biomass.

steppe, and there was a tendency (NS) for higher amounts in elevated than ambient CO_2 treatments (Fig. 1b). Interannual variability in crown biomass did not appear to follow any trend. After 5 yrs of exposure to the OTC experimental treatments, the depth distribution of root biomass was not significantly altered (Fig. 2).

Root C : N ratios were significantly altered by the CO_2 treatments ($P = 0.002$), with significant interannual variability ($P = 0.0008$) and CO_2 by year interaction ($P = 0.0001$; Table 2; Fig. 3). In 1998, the main effect was an increase in C : N ratios owing to the presence of open-top chambers

Table 2 Results of repeated measures analysis of variance for root C : N ratios (ash corrected), for 1998–2001, with CO_2 and Depth as main effects and Year as the repeated measure

Effect	DF	*P*-Value
CO_2	2	0.002
Depth	5	< 0.0001
$CO_2 \times$ Depth	10	0.4367
Year	3	0.0008
Year $\times$ CO_2	6	0.0001
Year $\times$ Depth	15	0.0007
Year $\times$ $CO_2 \times$ Depth	30	0.2703

(AC > NC, $P = 0.021$ and EC > NC, $P = 0.012$), with no CO_2 effect. In 1999, the chamber effect was overwhelmed by the elevated CO_2 effect, with EC > AC ($P = 0.0024$) and AC not different from NC. In 2000, the CO_2 treatment grew stronger, with EC > AC ($P = 0.0001$) but a significant chamber effect, opposite to that of 1998, was seen (AC < NC, $P = 0.011$). After 5 yrs of experimental treatment, in 2001, the maximum treatment effect on C : N ratios in roots was observed, with EC > AC ($P < 0.0001$), and AC < NC ($P = 0.014$). A significant interaction between CO_2 treatment and depth ($P = 0.004$) only became apparent in 2001; C : N in EC roots stayed relatively higher at deeper depths, in contrast to the pattern of increasing, then decreasing C : N ratios in NC roots (Fig. 4).

$\delta^{13}C$ of roots by species

We noted significant effects of CO_2 treatment and plant part (leaves, crowns, roots at 0–20, 20–45 and 45–75 cm) on $\delta^{13}C$ values of each species harvested at the end of the experiment. In general, leaves had more negative values than roots or crowns (Fig. 5). This effect was most noticeable in the EC treatment, because root and crown biomass accumulate over several years, whereas leaf biomass represents the current year's growth. Root $\delta^{13}C$ values (averaged from 0 to 45 cm) of the most abundant C_4 grass at the short grass steppe site, *B. gracilis*, were 1‰ higher than leaves in NC plots, 2‰ higher than leaves in AC plots, and 3.9‰ higher than leaves in EC plots (Fig. 5a). Root $\delta^{13}C$ values of the C_3, *P. smithii*, were 0.5‰ higher than leaves in NC and AC plots, and 6.4‰ higher than leaves in EC plots (Fig. 5b). Root $\delta^{13}C$ values of the C_3, *S. comata*, were 1.6‰ higher than leaves on NC plots, 1.4‰ higher than leaves on AC plots, and 3.4‰ higher than leaves on EC plots (Fig. 5c). Overall roots averaged 1.4 ± 0.9‰ heavier than leaves under ambient and nonchambered conditions, and this offset was used to estimate 'new' C input end member values (Table 3).

Differences in root $\delta^{13}C$ values between EC and NC monoliths were used to estimate the residence time for each grass species in the EC treatment (applying Equation (2) to the root

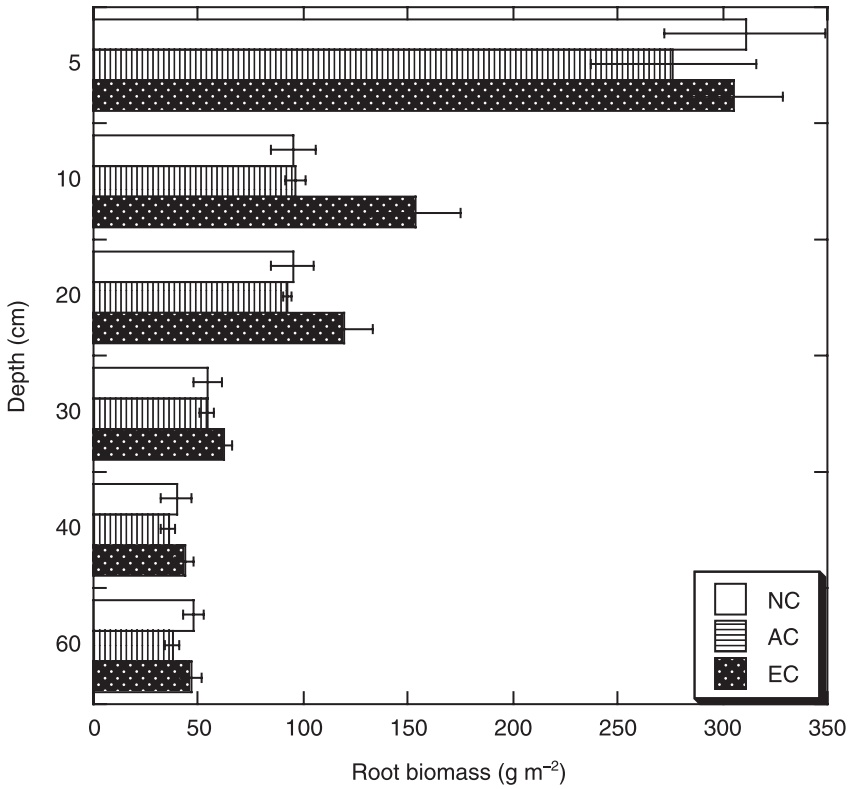

Fig. 2 Root biomass depth profiles from 2001, after 5 yrs of treatments. Nonchambered (NC), ambient CO_2 (AC) and elevated CO_2 (EC) chambers.

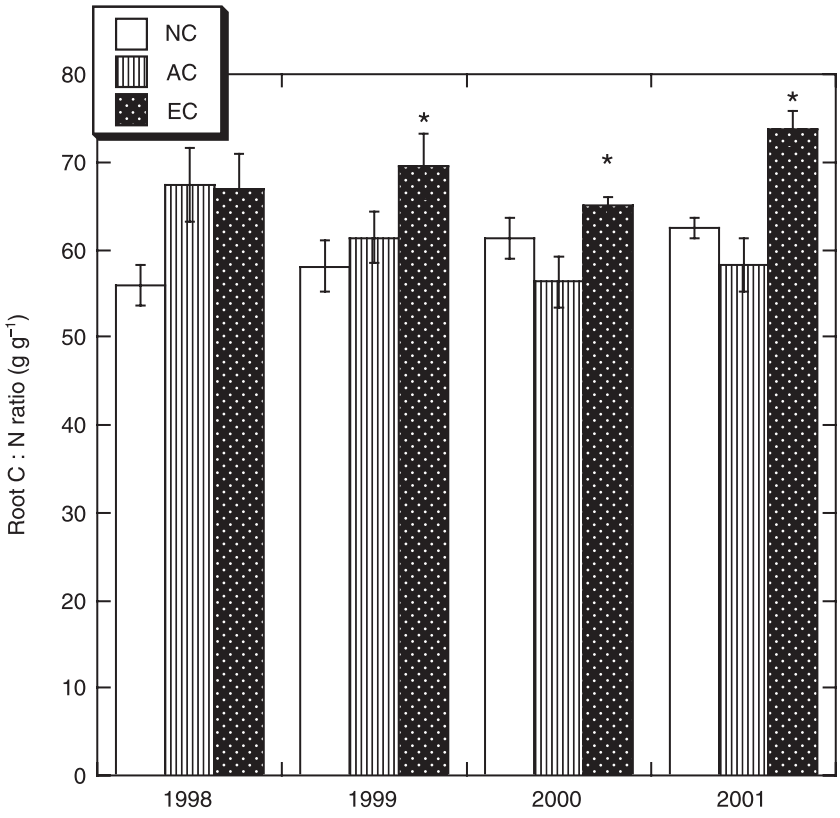

Fig. 3 Average root C : N ratios in the last 4 yrs of the experiment; average weighted by mass of soil in each depth increment. Nonchambered (NC), ambient CO_2 (AC) and elevated CO_2 (EC) chambers. Asterisks show years when EC was significantly different from AC ($P < 0.05$). Error bars were calculated as the SE of the mean.

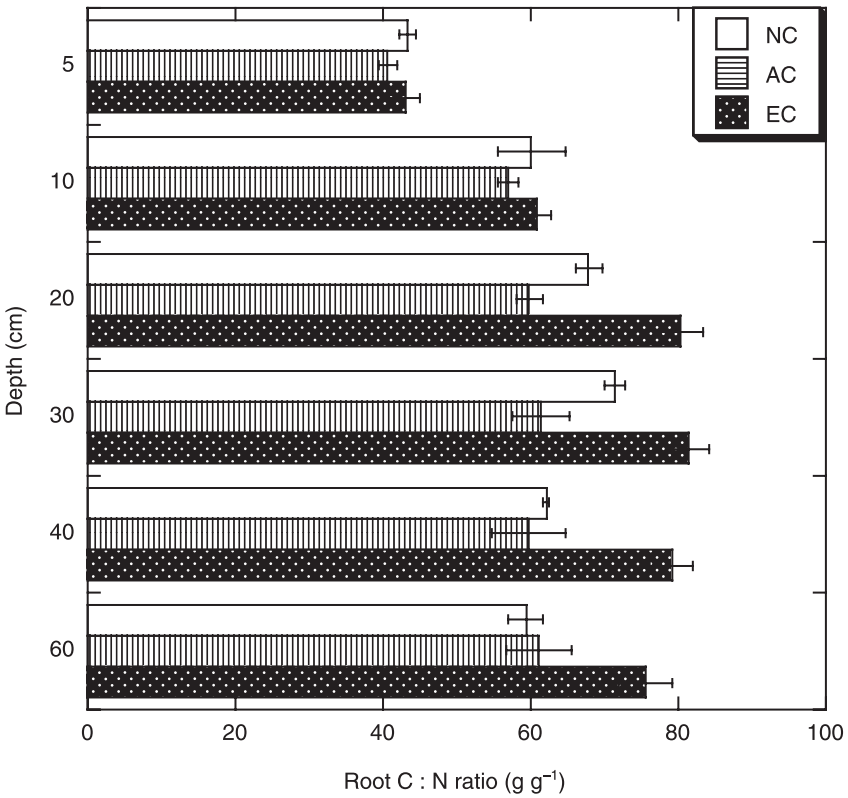

Fig. 4 Depth profile of root C : N ratios in 2001, after 5 yrs of experimental treatment. Nonchambered (NC), ambient CO_2 (AC) and elevated CO_2 (EC) chambers.

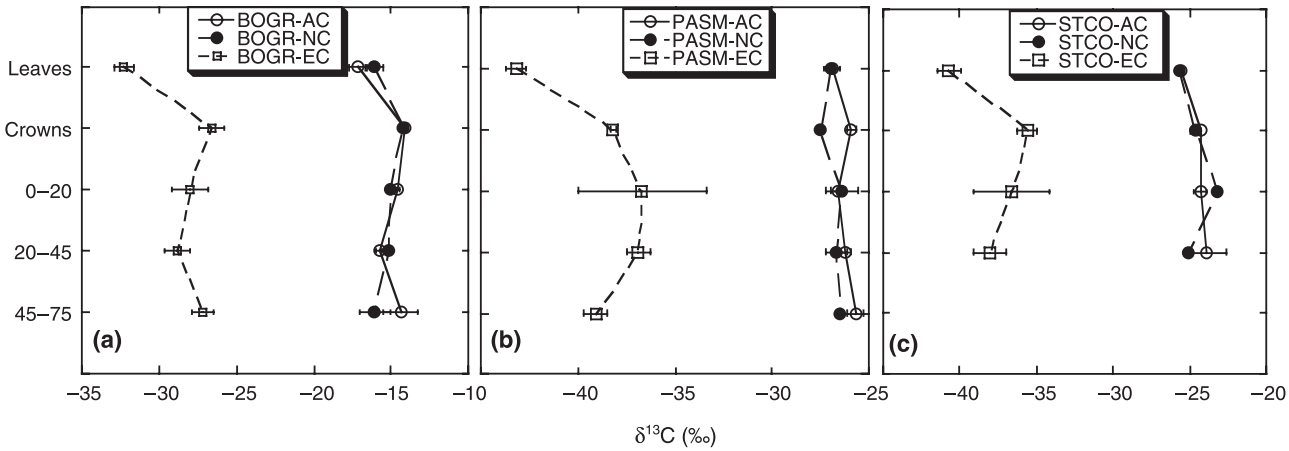

Fig. 5 $\delta^{13}C$ values in leaves, crowns, and roots at 3 depths after 5 yrs of experimental treatments in the three most abundant grasses. AC, ambient CO_2 chambers; NC, nonchambered control plots; EC, elevated CO_2 chambers. (a) $\delta^{13}C$ in the C_4 grass, *Bouteloua gracilis* (BOGR). (b) $\delta^{13}C$ in the C_3 grass, *Pascopyrum smithii* (PASM). (c) $\delta^{13}C$ in the C_3 grass, *Stipa comata* (STCO).

values). After 5 yrs of elevated CO_2, new roots made up 84% of *S. comata*, 63% of *P. smithii*, and 80% of *B. gracilis* roots in top 20 cm, corresponding to root residence times of approx. 6 yrs for *S. comata* and *B. gracilis* and approx. 8 yrs for *P. smithii*. In the 20–45 cm increment, 93% of *S. comata* roots were added during the 5-yr experiment (residence time 5.4 yrs) but similar proportions as in the top 20 cm were added for the other species.

Rhizodeposition

The $\delta^{13}C$ of 'new' C added annually to soils to 40-cm depth was estimated from the $\delta^{13}C$ values of C_3 and C_4 plants and their proportional cover (Table 3). The 'new' end-members in EC plots had very low $\delta^{13}C$ values because the tank gas added to the chambers to double the CO_2 concentration was depleted in ^{13}C (Pendall *et al.*, 2003), and also because the proportion

Table 3 Isotopic composition of C_3 and C_4 plants, the relative percent of C_3 biomass, and the $\delta^{13}C$ values of the 'new' C end-member

	C_4 ‰	C_3 ‰	% C_3 %	'New' $\delta^{13}C$ ‰
1997				
AC	−16.1 (0.5)	−26.5 (0.7)	49 (6)	−19.8 (1.3)
EC	−29.5 (0.4)	−40.1 (0.6)	61 (3)	−34.6 (1.2)
NC	−15.4 (0.4)	−26.6 (0.8)	57 (7)	
1998				
AC	−14.7 (0.4)	−26.9 (1.7)	70 (2)	−21.8 (2.0)
EC	−30.1 (0.1)	−41.3 (2.8)	79 (8)	−37.5 (3.0)
NC	−15.1 (1.4)	−27.1 (3.7)	72 (15)	
1999				
AC	−15.4 (0.2)	−25.2 (0.9)	72 (3)	−21.1 (0.6)
EC	−33.2 (0.8)	−42.4 (1.9)	81 (4)	−39.3 (2.1)
NC	−15.4 (0.3)	−26.5 (0.5)	67 (7)	
2000				
AC	−13.4 (0.9)	−24.0 (2.4)	63 (5)	−18.7 (2.8)
EC	−28.3 (1.0)	−32.8 (1.7)	77 (4)	−30.4 (2.2)
NC	−15.2 (1.0)	−26.0 (0.9)	58 (7)	
2001				
AC	−16.0 (0.1)	−26.9 (0.6)	53 (2)	−20.4 (1.1)
EC	−29.4 (0.7)	−38.1 (4.1)	64 (9)	−33.6 (4.3)
NC	−16.4 (0.1)	−25.2 (0.9)	50 (12)	

SE of the mean in parentheses.

of C_3 biomass increased over the experiment (Morgan *et al.*, 2004). Most of the interannual variability in the average new end-member value resulted from changes in C_3 versus C_4 plant biomass in all treatments; this proportion varied spatially and decreased in the last 2 yrs because of extremely dry conditions (Table 3). The average $\delta^{13}C$ of C_3 and C_4 plants in AC plots was slightly higher in 2000 than in the other years because a pulse-labelling experiment was conducted to evaluate short-term changes in exudation (Pendall *et al.*, unpublished data). A similar pattern was seen in EC plots because of an unintentional change in tank gas isotopic composition for a few weeks at the beginning of the 2000 growing season. Because 2000 was a very dry growing season, especially in spring, plant growth was limited and only a small portion of the total C was affected by these variations. Nonetheless, we accounted for the interannual shifts in isotopic composition of plant material and proportional biomass when calculating rhizodeposition.

Significant changes in the total mass of soil carbon attributable to elevated CO_2 were not found in this experiment (see Appendix). We therefore relied on changes in the stable C isotopic composition of SOM to improve the detection of new C inputs. In EC soils, C was added to all depth intervals every year, and in AC soils, detectable C was added in at least two depth intervals every year (Appendix). When summed up over the top 40 cm, in 1997, rhizodeposition contributed nearly 200 g C m^{-2} in both AC and EC treatments, and from 1998 to 2001, between 65 and 100 g C m^{-2} yr^{-1} was added to EC soils, and 26–40 g C m^{-2} yr^{-1} was added to AC soils (Fig. 6).

Discussion

Root biomass and C : N ratios

Elevated CO_2 stimulated above-ground biomass production in the short grass steppe OTC experiment by an average of

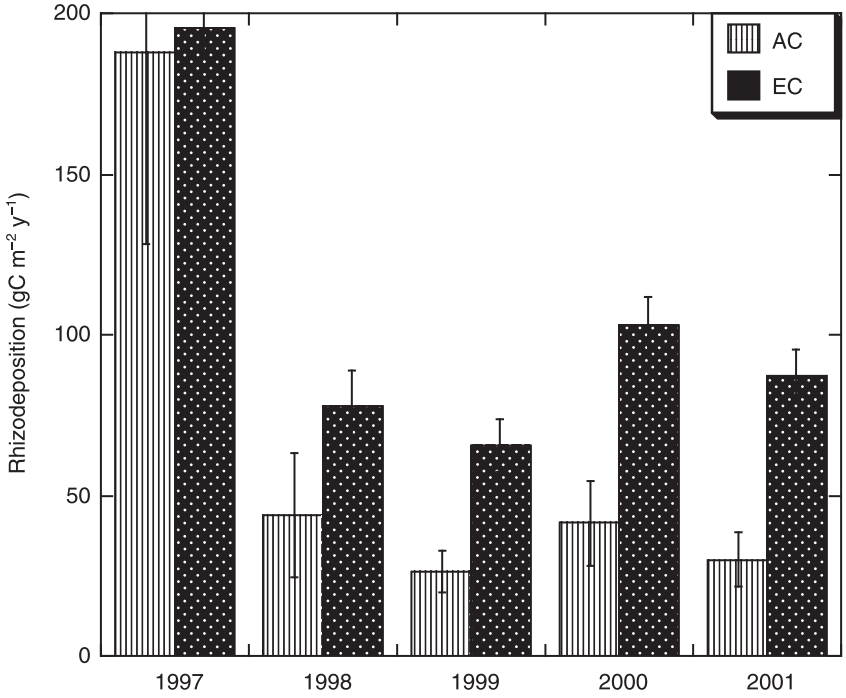

Fig. 6 Annual rates of rhizodeposition, or 'new' soil C inputs, in the top 40 cm of soil over the 5-yr experiment. Error bars represent cumulative errors in isotopic composition of end members and mixtures (Phillips & Gregg, 2001), and include corrections for spatial covariance among values at different depths in the soil (see the Materials and Methods section).

33% over the 5 yrs, and a chamber effect which lengthened the growing season stimulated above-ground biomass production in AC relative to NC plots by a similar amount (Morgan *et al.*, 2004). After 5 yrs, we found that elevated CO_2 stimulated root biomass by 23% in the top 60 cm (cylinder data). Root monoliths excavated to 75-cm depth and sampled at coarser depth intervals showed a trend of increased biomass under elevated CO_2 (NS). Elevated CO_2 has often stimulated root biomass production, especially in grasslands (Fitter *et al.*, 1997; Jastrow *et al.*, 2000; Higgins *et al.*, 2002), but has sometimes had no effect or even reduced root biomass (Kandeler *et al.*, 1998; Arnone *et al.*, 2000).

The presence or absence of root biomass stimulation, and depth patterns of biomass enhancement are probably related to changing resource availability under elevated CO_2. In our experiment, root biomass increased at all depths, but especially in the 5–10 cm increment. Root biomass increased in the top 6-cm of a calcareous grassland but decreased below 12 cm-depth under elevated CO_2 (Arnone *et al.*, 2000). Soil water content in the short grass steppe OTC experiment increased significantly, especially lower in the profile (Nelson *et al.*, 2004). When soil resource availability is increased by elevated CO_2, root production might be expected to decrease (Kandeler *et al.*, 1998); however, increased water availability was probably responsible for a portion of the biomass enhancement both above- and below-ground in this water-limited ecosystem (Morgan *et al.*, 2001, 2004).

Because the roots of most short grass steppe species are relatively long-lived (5–7 yrs based on ^{14}C labelling; Milchunas & Lauenroth, 2001), we expected the response of our system to be slow. We did not expect to see the strong effect in 1998, after only 2 yrs of treatment, and we do not have a good explanation for the decreasing root biomass in the last 3 yrs of the experiment. A severe drought began in 2000 and lasted through 2001, but this would not explain the decrease beginning in 1999. Minirhizotron and root-ingrowth core data from this experiment should reveal details of inter- and intra-seasonal dynamics in root production and its possible relationship to climate patterns (D. Milchunas, unpublished data). We believe that the insertion of the steel cylinders did not have a long-term effect on productivity because root biomass in cylinders in 2001 was similar to that in monoliths.

Decreased N concentrations are associated with lower root mortality or turnover rates, and thus may eventually reduce decomposition rates (reviewed in Pendall *et al.*, 2004a). After 5 yrs of elevated CO_2 on the short grass steppe, root C : N ratios increased by 26% when averaged through the whole 60-cm profile, but the increase was only about 6% in the top 10 cm, and about 33% between 10 and 40-cm depth. This depth by CO_2 interaction appeared only in 2001, suggesting that the changing plant community composition, driven by elevated CO_2 (Morgan *et al.*, 2004), was differentially affecting rooting depth. It seems likely that different rhizosphere microbial communities will evolve at different depths, driven by depth-dependent quality of rhizodeposits, in turn affecting C turnover and nutrient cycling.

Root C : N ratios were not significantly altered at the end of an 8-yr-long tallgrass prairie OTC experiment, but particulate organic matter (POM) C : N in the top 5 cm did increase under elevated CO_2 (Jastrow *et al.*, 2000). The C : N ratios of POM, derived mainly from roots in experimental ryegrass swards (*Lolium perenne*), only increased under elevated CO_2 if soil N availability was low (Loiseau & Soussana, 1999). Decomposition rates were slowed as the lower quality litter entered older SOM fractions. Older SOM decomposition rates were stimulated by elevated CO_2 in the third and fourth years of the short grass steppe OTC experiment; apparently the differences in C : N we observed were insufficient to suppress decomposition rates at that point in the experiment (Pendall *et al.*, 2003). However, the lower root N concentrations we observed may help explain lower specific root respiration in 1999 (Fitter *et al.*, 1997; Pendall *et al.*, 2003).

Root residence times estimated from changing isotopic composition must be interpreted cautiously, but our estimates in the EC treatment are similar to radiocarbon labelling estimates of 5–7 yrs on bulk roots in a nearby shortgrass steppe study under ambient CO_2 conditions (Milchunas & Lauenroth, 2001). Isotope dilution by pretreatment nonstructural carbohydrates may cause root longevity to be overestimated, which may have been a factor in our long-lived root systems. We emphasize that our estimates came from the EC treatment only, and root longevity may be underestimated because root production was higher than in AC or NC treatments (Luo, 2003). However, the increased root C : N ratios on EC plots may counteract the effect of increased production by slowing root mortality. The species-specific response we noted, with *S. comata* having the shortest root residence time, likely reflects stimulated root growth in tandem with the above-ground biomass response of this species to elevated CO_2; the other species in this study were not stimulated (Morgan *et al.*, 2004). Large differences in root longevity between loblolly pine (*P. taeda*) and sweetgum (*Liquidambar styraciflua*) growing under elevated CO_2 also point to the need for careful assessment of species effects in global change research (Matamala *et al.*, 2003).

Rhizodeposition ('new' soil C inputs) and implications for altered soil C storage

Our isotopic approach defined rhizodeposition to be derived from recent photosynthate, fixed within each growing season. The isotopically labelled C accumulated over the 5 yrs experiment, but we evaluated inputs for each year. Root exudates, turnover of fine roots, and root crowns produced within the growing season may all contribute to rhizodeposition as measured by stable isotopes. In our study, endomycorrhizas were inherently included in the analysis of root C and N, and may have been partly responsible for the isotopic offset

between leaves and roots. Pulse labelling studies may find most rhizodeposition allocated to root exudation, but continuous labelling studies such as ours are likely to quantify C pools that span a range of qualities with different decomposition dynamics (Paterson *et al.*, 1997). As with most below-ground process studies, quantitative and qualitative responses of rhizodeposition to elevated CO_2 are method-dependent (Pendall *et al.*, 2004a).

In the initial year of experimental treatment in a Swiss grassland, new C inputs (determined from a continuous ^{13}C label) were 210 g m^2 yr^{-1} in the top 10-cm of soil (Niklaus *et al.*, 2001). Initially rapid rates of new soil C inputs were also found in a Free-Air CO_2 Enrichment (FACE) study on *L. perenne* and *Trifolium repens* pastures (Van Kessel *et al.*, 2000). In both these studies, relatively high input rates were very nearly balanced by root turnover after the first year, because root residence times were < 2 yrs. In our study, the high input rates during the first year may have resulted from reduced soil moisture inside the chambers, which was subsequently remedied. This chamber effect in 1997 likely resulted in increased root mortality in both chambered treatments, some of which was distinguished by stable isotopes as rhizodeposition. Possibly, insertion of the cylinders contributed to variable root biomass and rhizodeposition dynamics in the first year or two. Nonetheless, elevated CO_2 increased rates of rhizodeposition for the last 4 yrs of our study; rhizodeposition was roughly doubled in EC compared to AC treatments, averaging 83 ± 16 versus 35 ± 9 g C m^{-2} yr^{-1} over the last 4 yrs (*t*-test, $P = 0.006$).

Although the current study does not specifically address the fate of the rhizodeposits added under elevated CO_2, widening C : N ratios of root biomass suggest that root turnover and decomposition rates will decrease, especially at depth. In a short-term microcosm study with *L. perenne*, radiocarbon labelling showed significantly increased rhizodeposition under elevated CO_2, and the proportion of this substrate incorporated into microbial biomass was reduced, indicating that microbes preferred utilizing older SOM with higher C : N ratios (Paterson *et al.*, 1999). Cardon *et al.* (2001) suggested that labile rhizodeposits were preferred by microbes over older SOM, at least when ample N was available, in an annual grassland elevated CO_2 experiment with mesocosms and a transplanted C_4 soil. In the shortgrass steppe OTC experiment, N appeared to be limiting enough to microbes that 'mining' and mineralization of older SOM occurred, at least during the first 3–4 yrs (Pendall *et al.*, 2003).

Whether stimulation of rhizodeposition by elevated CO_2 will result in a net accumulation of C in soils depends on whether pre-existing SOM is simply replaced by the new inputs. In the shortgrass steppe OTC experiment, stable isotopes provided constraints on the new C inputs as well as old C losses in both EC and AC treatments, and showed that net ecosystem production (NEP) did not change in 1999, a relatively wet year, as inputs balanced losses (Pendall *et al.*, 2004b). This grassland appears to maintain a C balance close to steady state under elevated CO_2, as decomposition increased nearly as much as rhizodeposition, which explains why total soil C pools did not change over 5 yrs of treatments.

Advantages and limitations of stable isotope techniques

Although the ^{13}C approach has considerable utility and potential for quantifying C fluxes from plants, C inputs, and turnover rates of SOM (Leavitt *et al.*, 1994, 2001; Paterson *et al.*, 1997), certain assumptions must be met for appropriate interpretation of the results. For instance, the end-members must be well characterized, and their $\delta^{13}C$ values should remain reasonably constant over space and time. In our case, we characterized spatial and temporal variations in the 'new' end-member, adjusted for a small offset between above- and below-ground $\delta^{13}C$ values, and also measured 'old' end-member values from NC soils annually. The main limitation for this study was partitioning new C additions on the AC plots, because isotopic end-members were only about 4‰ apart; no new additions were detectable in nearly 20% of the cases (see Appendix).

To our knowledge, this is the first paper using $\delta^{13}C$ to calculate soil C inputs which has accounted for all measurable sources of error; we used a Taylor-series approach to accumulate the errors in the end-members and the mixtures (Phillips & Gregg, 2001), and furthermore explicitly accounted for covariance among soil depths in $\delta^{13}C$ and C content values. The main uncertainties in our 'new' end-member $\delta^{13}C$ values stemmed from unknown contributions of leaf, root, and crown biomass to the new C pools, and how this varied with depth in the soil. Additional unknown uncertainties may result from differential decomposition dynamics of C_3 and C_4 plants, which would affect the $\delta^{13}C$ of litter as it decomposes (presumably this may include roots; Wedin *et al.*, 1995). Finally, root and leaf biomass is mainly structural, which may have a different $\delta^{13}C$ value than metabolic C, while rhizodeposition is considered by some to include mainly metabolic C (Paterson *et al.*, 1997). Our approach using $\delta^{13}C$ values from bulk soil C and plant tissues included a range of compounds of varying quality. The accuracy of the ^{13}C method will be improved as we learn more about the physiochemical nature of C inputs to soil and how they change isotopically during decomposition.

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Appendix Bulk soil C content and isotopic composition, and new C input rate

Trt, year	Depth (cm)	Total soil C (gm^{-2})	$\delta^{13}C$ (‰)	C inputs (gm^{-2})
AC	0–5	617 (61)	–17.7 (0.9)	72 (56)
AC	5–10	520 (38)	–16.5 (0.8)	0
AC	10–20	842 (24)	–15.5 (0.1)	30 (9)
AC	20–30	909 (29)	–15.1 (0.1)	53 (14)
AC	30–40	737 (66)	–14.6 (0.3)	33 (14)
EC	0–5	645 (129)	–18.8 (0.2)	49 (5)
EC	5–10	540 (90)	–17.8 (0.1)	37 (3)
EC	10–20	857 (115)	–16.2 (0.2)	36 (2)
EC	20–30	864 (61)	–15.8 (0.3)	45 (3)
EC	30–40	702 (88)	–15.2 (0.4)	29 (2)
NC	0–5	545 (83)	–17.4 (1.0)	
NC	5–10	526 (51)	–16.6 (0.7)	
NC	10–20	881 (95)	–15.4 (0.3)	
NC	20–30	816 (105)	–14.8 (0.4)	
NC	30–40	666 (110)	–14.4 (0.4)	
1998				
AC	0–5	527 (44)	–17.7 (0.5)	0
AC	5–10	569 (60)	–16.5 (0.5)	0
AC	10–20	882 (24)	–15.2 (0.6)	0
AC	20–30	837 (44)	–14.9 (0.6)	23 (7)
AC	30–40	778 (121)	–15.1 (0.4)	65 (18)
EC	0–5	542 (74)	–19.5 (0.5)	42 (7)
EC	5–10	561 (32)	–18.4 (0.3)	39 (6)
EC	10–20	906 (18)	–16.1 (0.6)	29 (4)
EC	20–30	899 (54)	–15.7 (0.3)	36 (5)
EC	30–40	817 (102)	–14.8 (0.2)	10 (1)
NC	0–5	571 (115)	–17.8 (0.9)	
NC	5–10	591 (66)	–16.9 (0.3)	
NC	10–20	869 (19)	–15.4 (0.4)	
NC	20–30	914 (130)	–14.7 (0.2)	
NC	30–40	698 (97)	–14.5 (0.2)	
1999				
AC	0–5	488 (44)	–18.4 (0.3)	18 (4)
AC	5–10	510 (39)	–17.0 (0.3)	0
AC	10–20	872 (39)	–15.7 (0.1)	25 (3)
AC	20–30	877 (47)	–14.9 (0.2)	35 (3)
AC	30–40	857 (118)	–15.1 (0.3)	0
EC	0–5	522 (138)	–19.9 (0.3)	41 (4)
EC	5–10	470 (110)	–18.8 (0.3)	35 (3)
EC	10–20	891 (62)	–16.8 (0.1)	46 (4)
EC	20–30	908 (55)	–15.9 (0.2)	46 (4)
EC	30–40	798 (102)	–16.0 (0.3)	29 (3)
NC	0–5	540 (93)	–18.3 (0.2)	
NC	5–10	596 (89)	–17.2 (0.5)	
NC	10–20	924 (45)	–15.6 (0.4)	
NC	20–30	963 (113)	–14.6 (0.2)	
NC	30–40	824 (113)	–15.1 (0.4)	
2000				
AC	0–5	618 (103)	–18.3 (0.1)	0
AC	5–10	596 (52)	–17.1 (0.3)	28 (9)
AC	10–20	962 (28)	–15.9 (0.2)	51 (9)
AC	20–30	1005 (101)	–15.3 (0.1)	43 (4)
AC	30–40	891 (141)	–14.9 (0.2)	44 (3)
EC	0–5	616 (112)	–20.8 (0.4)	119 (8)
EC	5–10	581 (66)	–18.9 (0.1)	79 (3)
EC	10–20	912 (53)	–17.2 (0.2)	92 (3)
EC	20–30	910 (37)	–16.3 (0.2)	66 (2)
EC	30–40	784 (117)	–15.8 (0.1)	55 (1)
NC	0–5	573 (47)	–18.5 (0.6)	
NC	5–10	573 (61)	–17.0 (0.4)	

Appendix *continued*

Trt, year	Depth (cm)	Total soil C (gm^{-2})	$\delta^{13}C$ (‰)	C inputs (gm^{-2})
NC	10–20	900 (60)	–15.8 (0.4)	
NC	20–30	917 (60)	–15.2 (0.3)	
NC	30–40	810 (100)	–14.7 (0.2)	
2001				
AC	0–5	603 (123)	–18.1 (0.3)	0
AC	5–10	574 (33)	–17.1 (0.4)	53 (7)
AC	10–20	1056 (108)	–16.2 (0.4)	84 (10)
AC	20–30	1039 (79)	–15.4 (0.4)	14 (1)
AC	30–40	1008 (152)	–15.0 (0.4)	0
EC	0–5	593 (47)	–21.0 (0.3)	106 (7)
EC	5–10	589 (32)	–19.7 (0.1)	101 (5)
EC	10–20	1022 (67)	–17.6 (0.2)	102 (3)
EC	20–30	947 (71)	–16.9 (0.2)	82 (2)
EC	30–40	797 (110)	–16.3 (0.3)	46 (1)
NC	0–5	758 (71)	–18.2 (0.6)	
NC	5–10	630 (57)	–16.8 (0.3)	
NC	10–20	1090 (59)	–15.8 (0.4)	
NC	20–30	1037 (101)	–15.3 (0.3)	
NC	30–40	909 (87)	–15.3 (0.5)	